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Figure 1

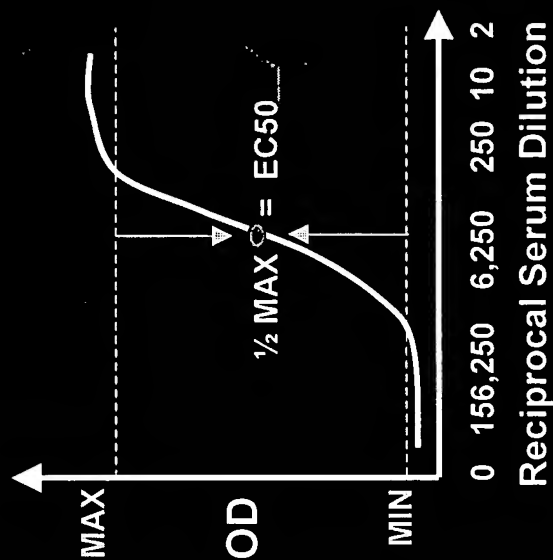
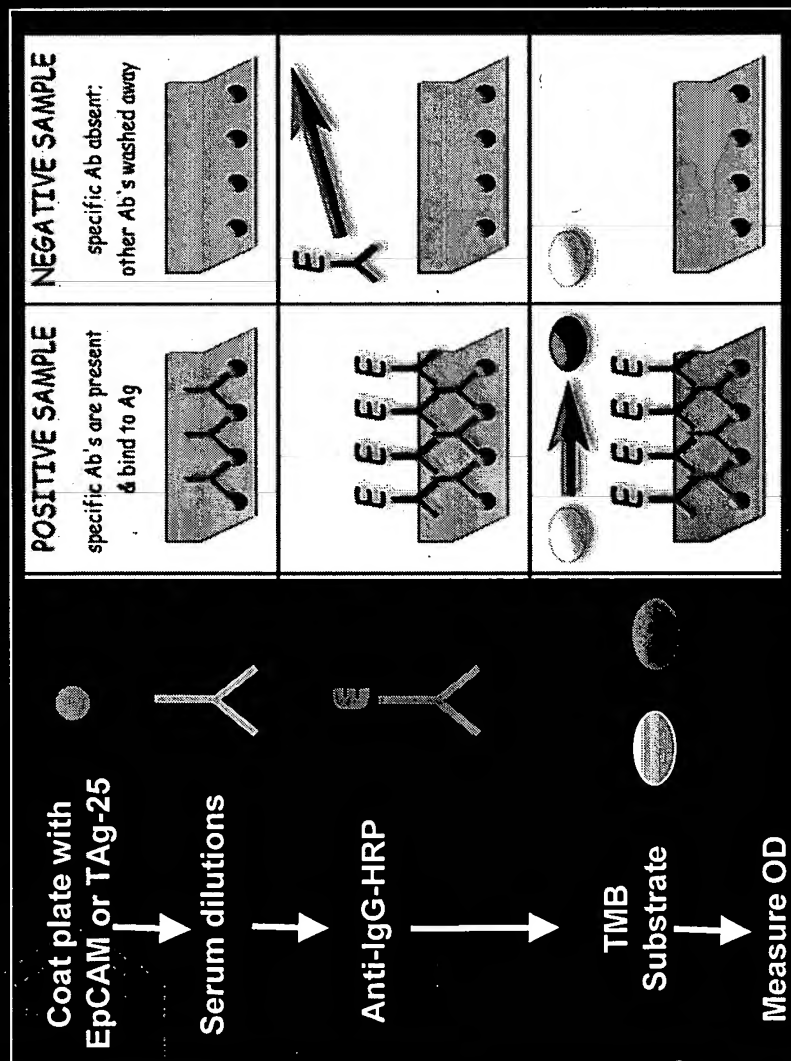


Figure 2

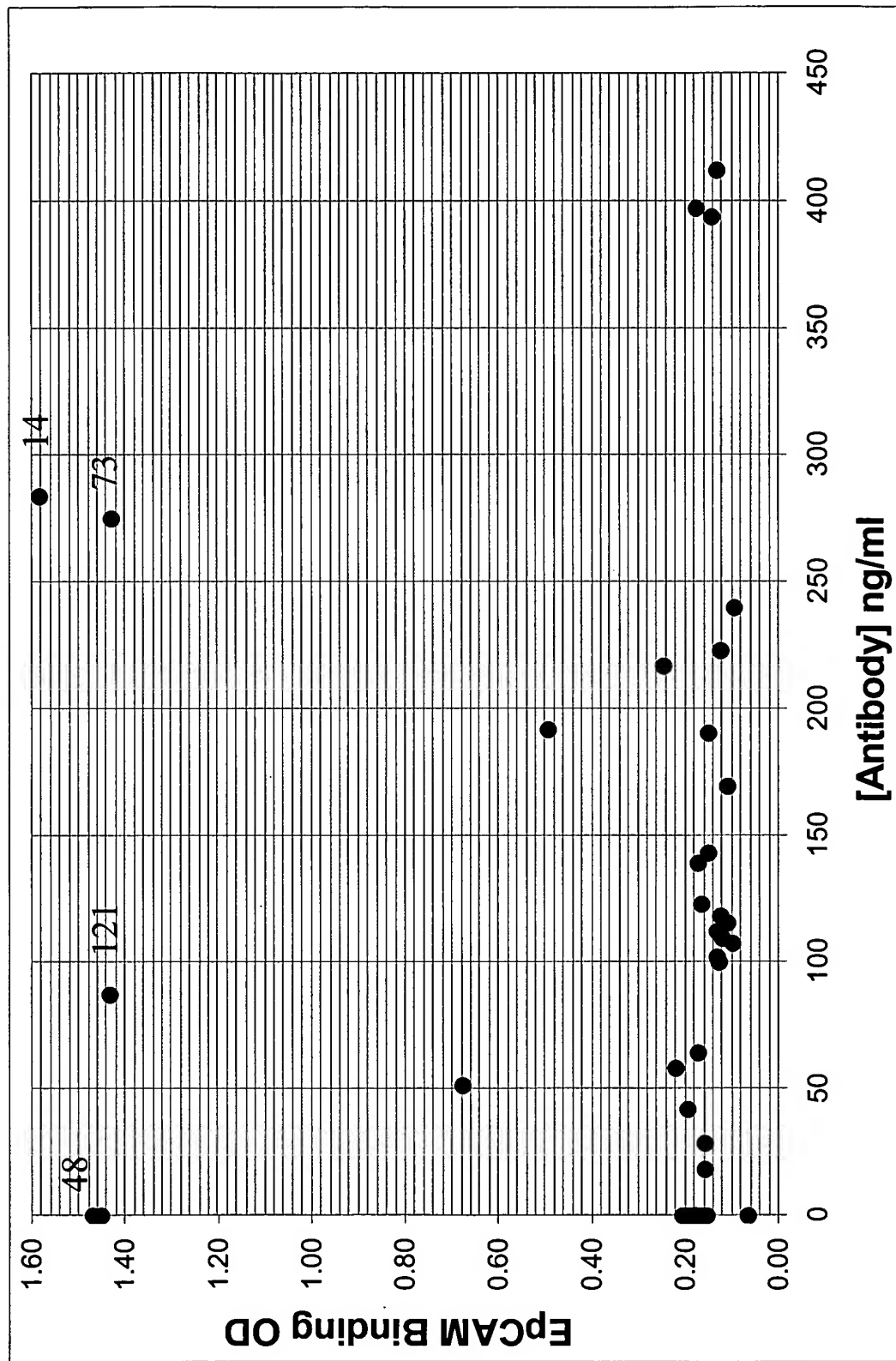


Figure 3

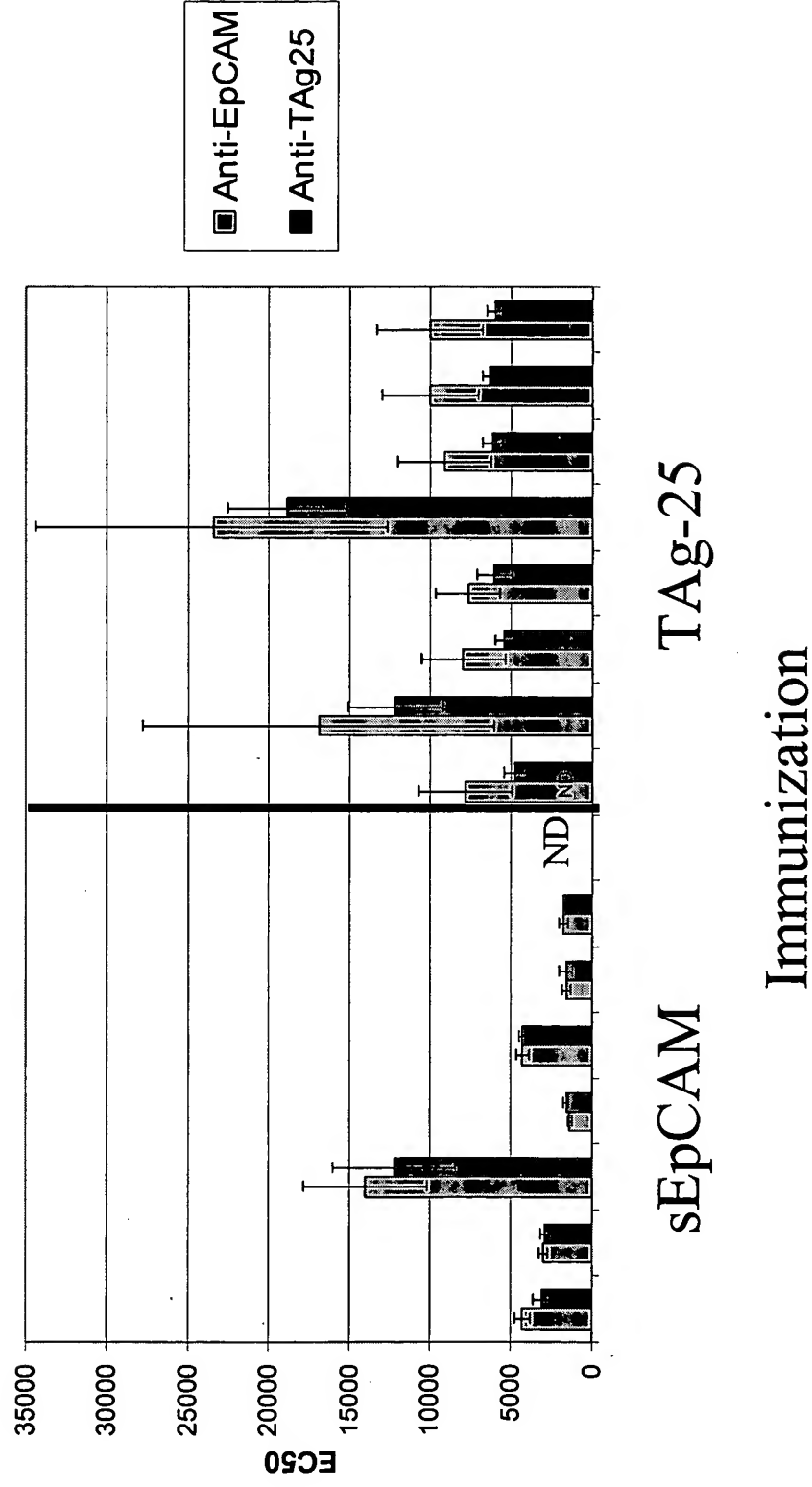


Figure 4

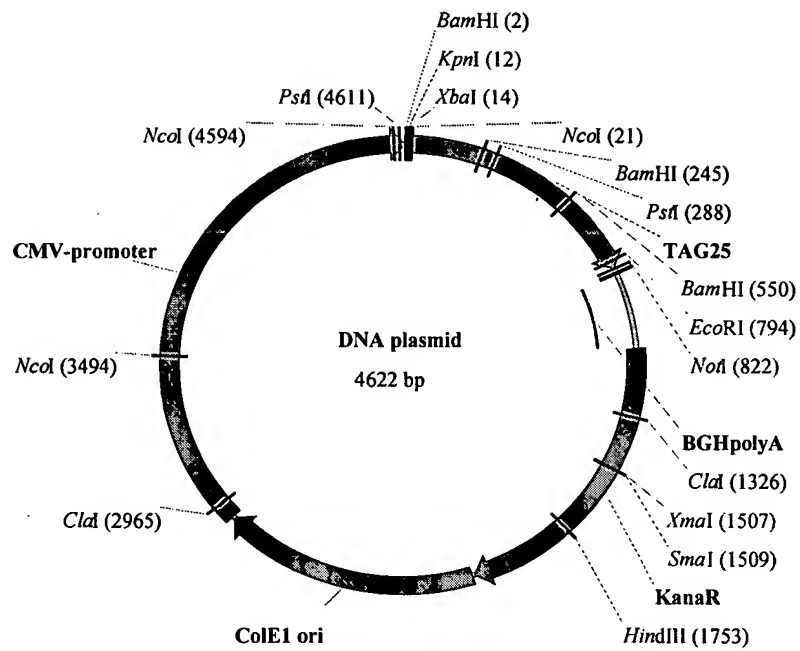


Figure 5

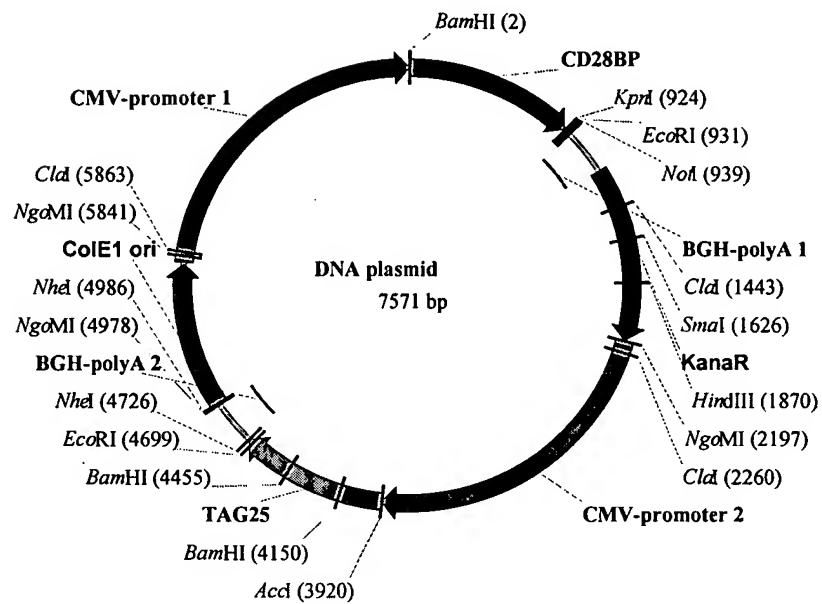
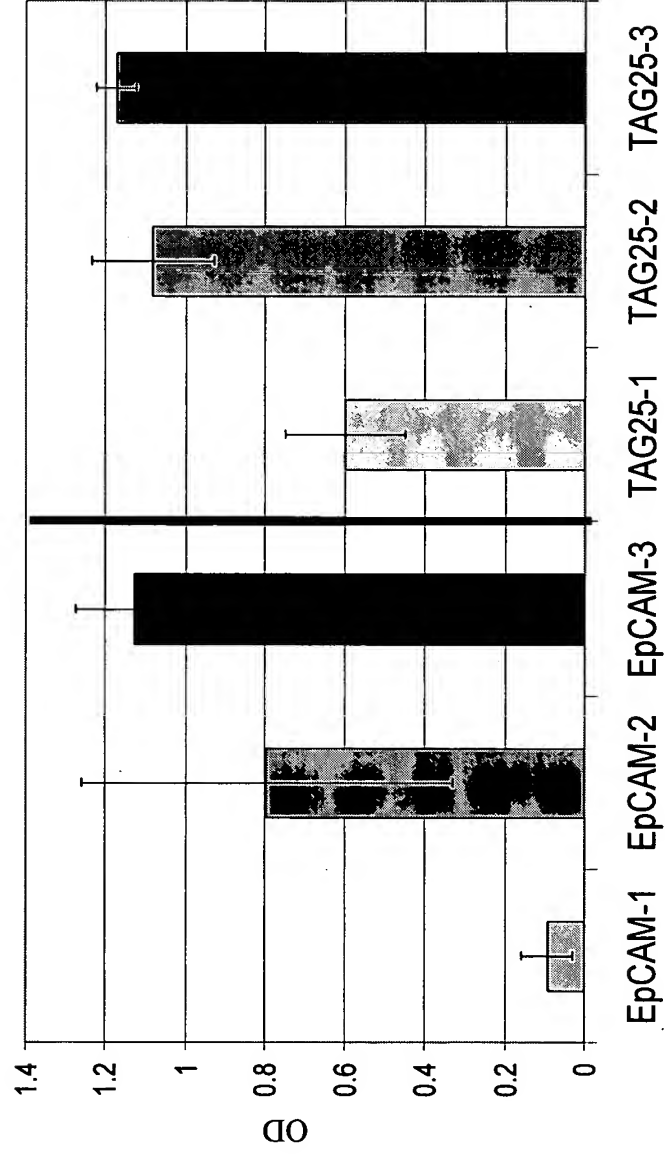


Figure 6



Figure 7



Immunitization

Figure 8

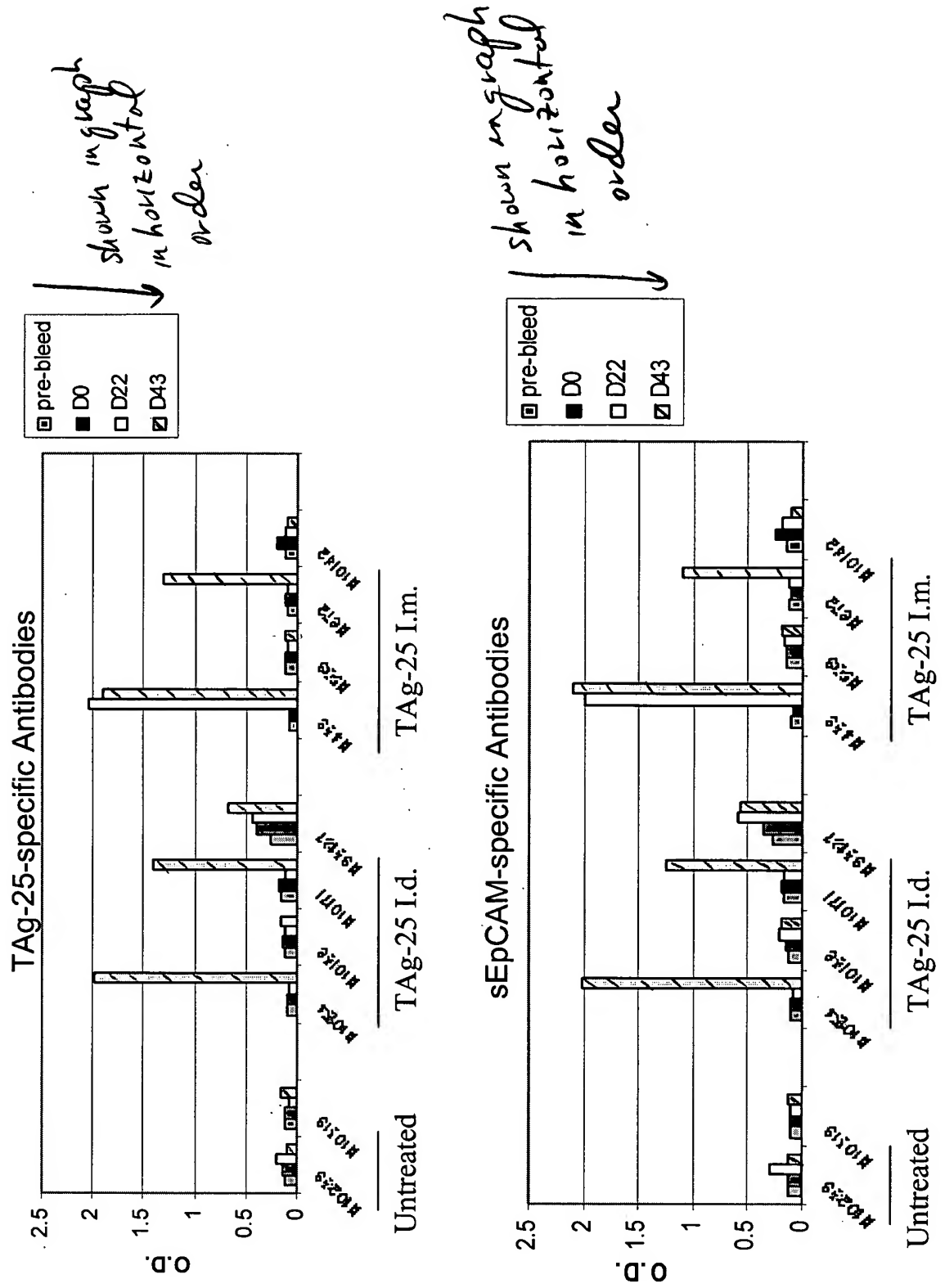


Figure 9A

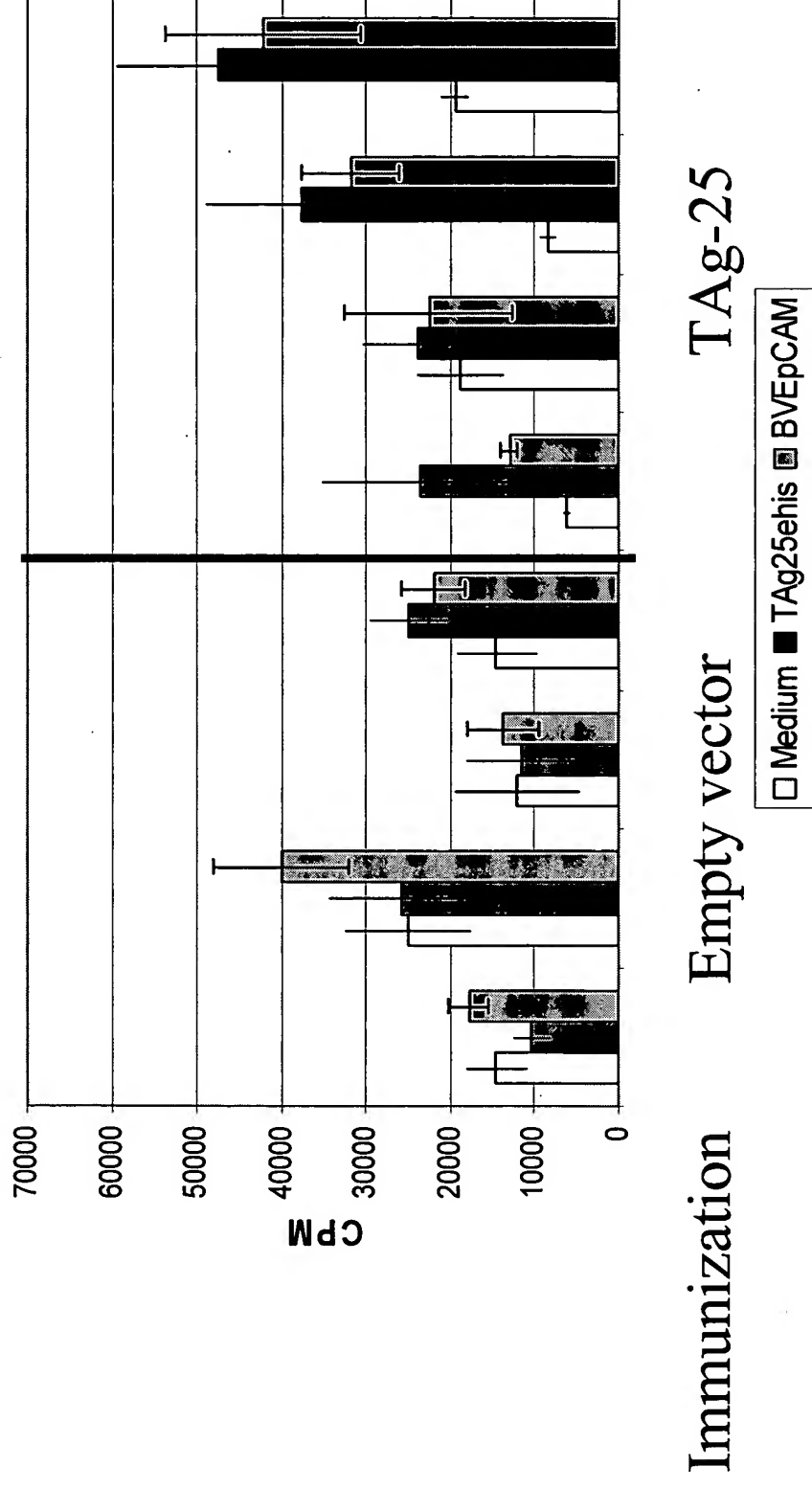
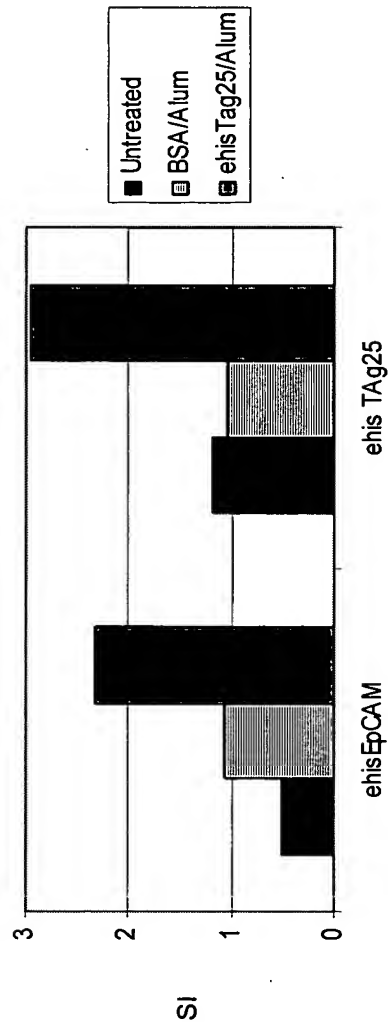


Figure 9B

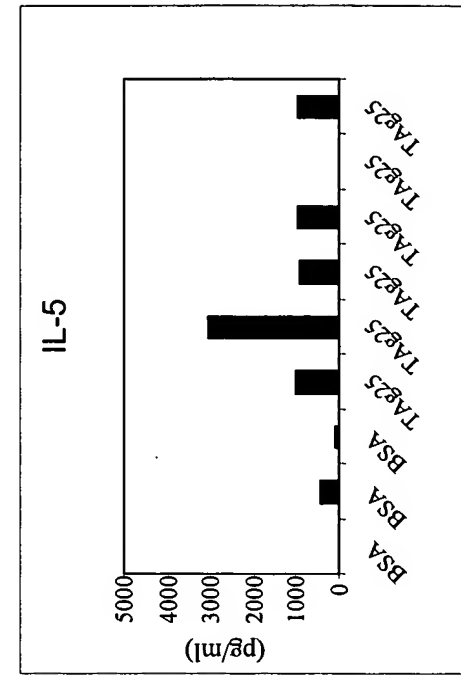
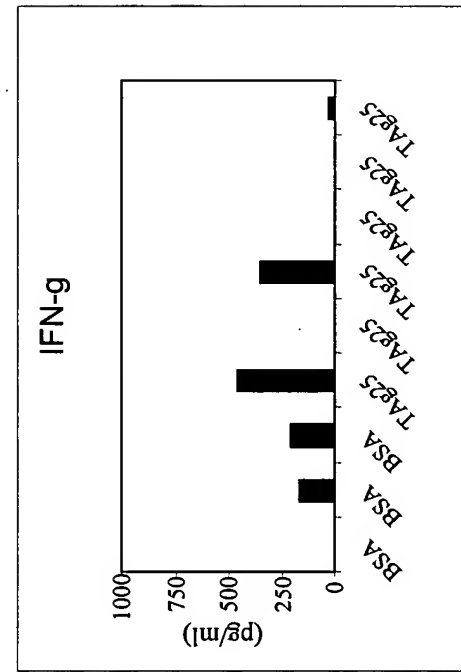
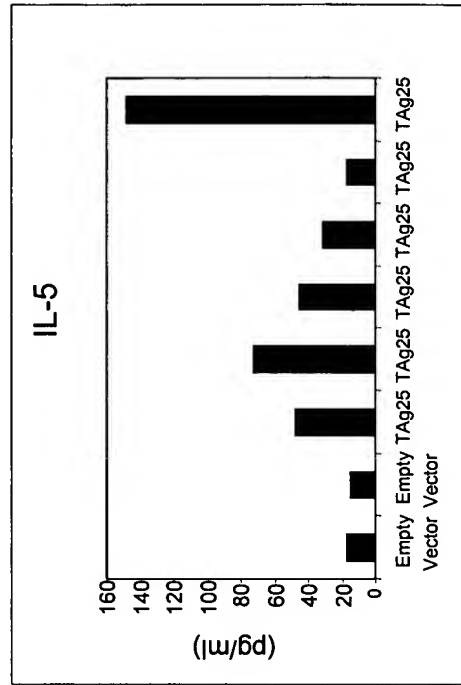
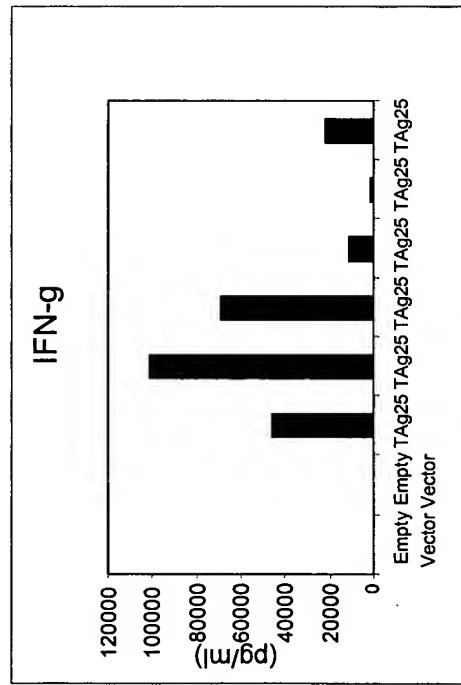
Protein Vaccine:



Stimulation in vitro

↓ shown in graph in longitudinal order

A



Immunization

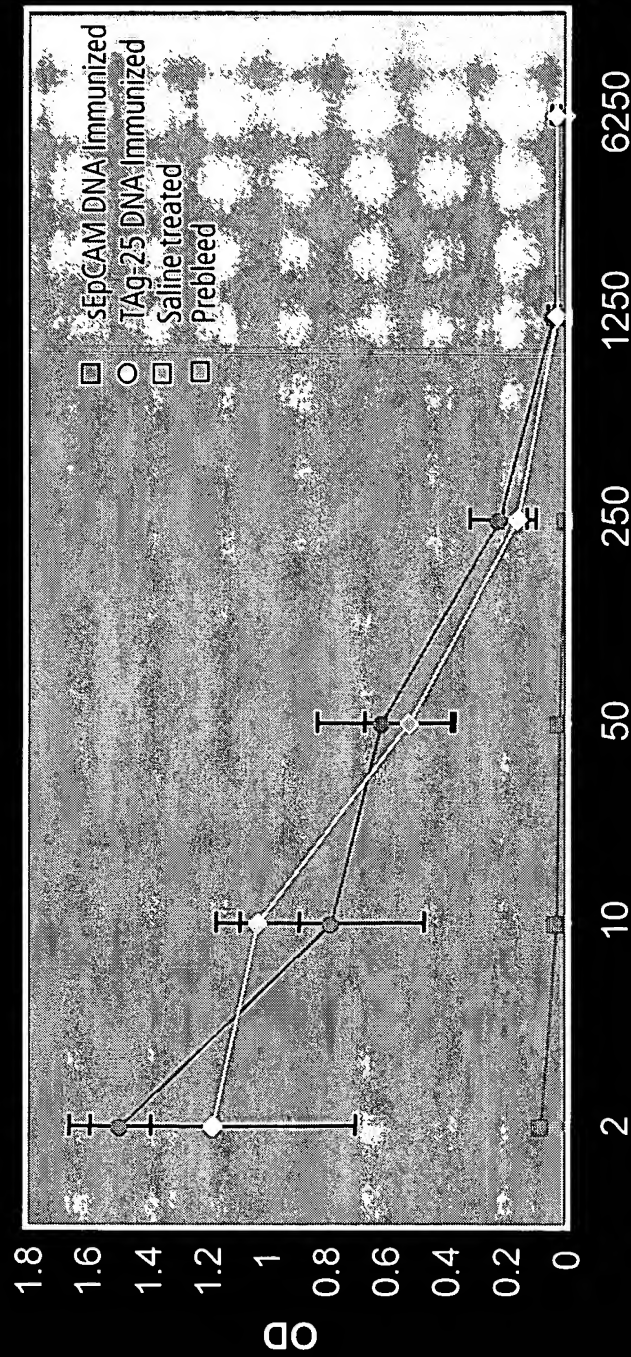
Figure 11

DNA Treatment	Route	Pre-bleed		1st DNA		2nd DNA		3rd DNA		4th DNA	
		Responders to EpCAM	Responders to TAG25	Responders to EpCAM	Responders to TAG25	Responders to EpCAM	Responders to TAG25	Responders to EpCAM	Responders to TAG25	Responders to EpCAM	Responders to TAG25
Saline	im	0	0	0	0	0	0	0	0	0	0
EpCAM	id	0	0	0	0	1	1	3	3	3	3
EpCAM	im	0	0	1	0	2	2	2	2	2	2
TAG25	id	0	0	0	0	0	0	3	3	3	3
TAG25	im	0	0	0	1	1	1	4	4	4	4

Monkeys were designated to be responders if their serum contained antibody levels that gave an EC50 of ≥ 5 or an OD value of ≥ 0.5 at a 1/10 serum dilution by ELISA for either EpCAM or TAG25

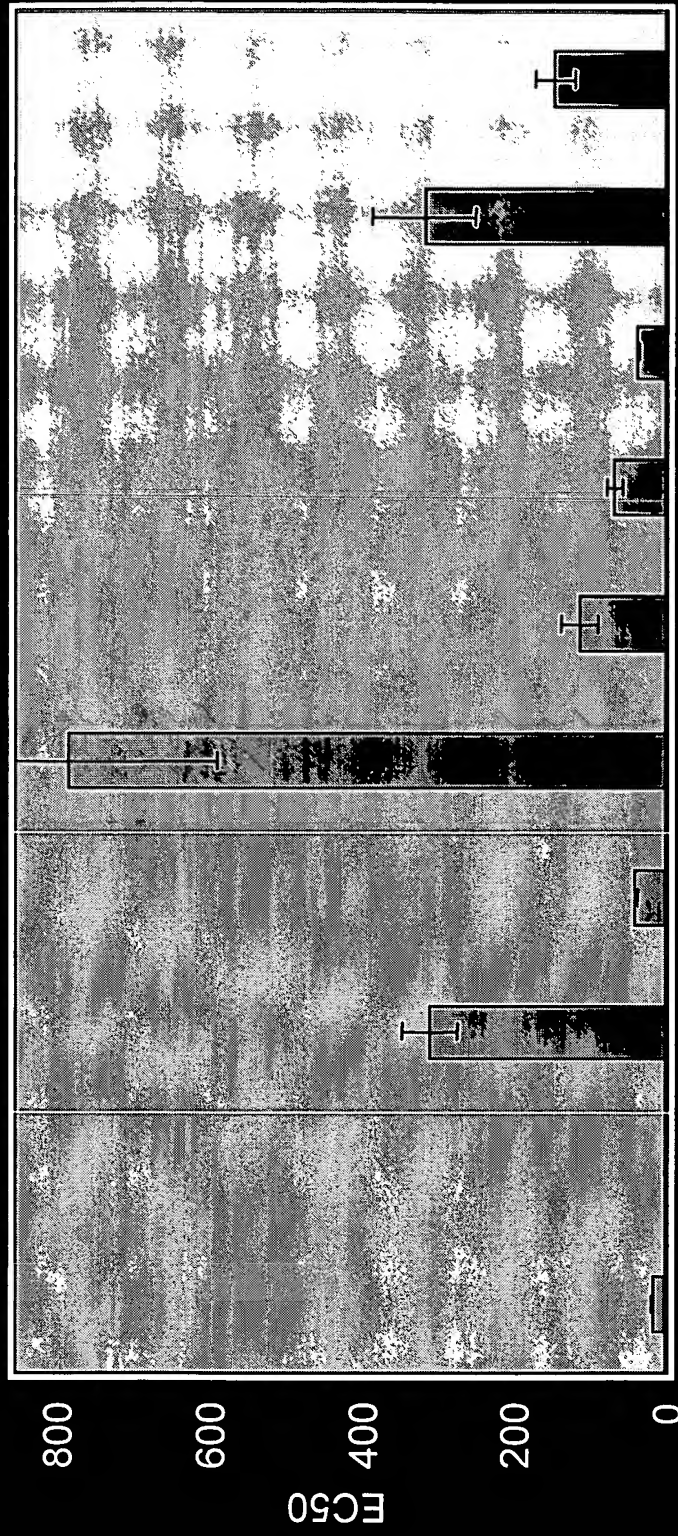
The number of responders is out of a total maximum of 4 for EpCAM or TAG25 treated groups or 2 for the saline treated group

Figure 12



Reciprocal Serum Dilution after 4th immunization with DNA vector (1 mg/per dose)

Figure 13



DNA →
immunization

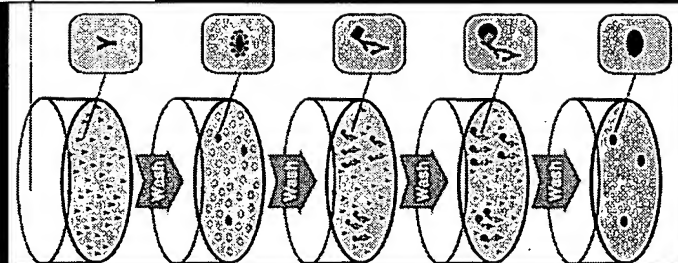
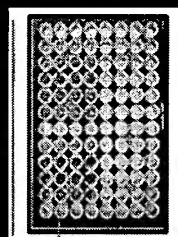
sEpCAM TAG-25 TAG-25 TAG-25 sEpCAM sEpCAM

DNA (delivery of
1mg DNA vector 4
times)

Protein (delivery of
empty control vector
followed by delivery of
100 ug TAG-25 protein
2 times)

DNA + Protein Boost
(delivery of 1mg DNA vector/4 times followed by
delivery of 100 ug TAG-25 protein 2 times)

Figure 15



- Preactivate PBMCs +/- antigen in tubes (2x10⁶/ml) for 16-24hr
- Coat PVDF membrane with capture anti-IFN γ Ab
- Block

• Add cells in quadruplicates

• Biotinylated capture Ab

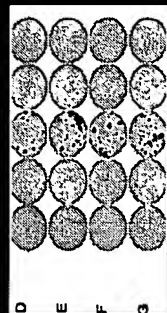
• Streptavidin HRP

• Substrate AEC

Readout: IFN γ Spot Forming Cells

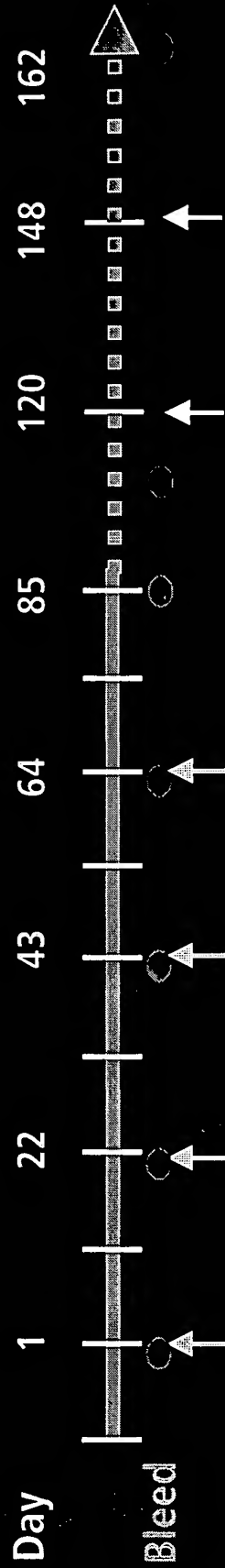


Elispot reader system
 (Carl Zeiss) with KS
 Elispot Software 4.3



Positive Negative

Figure 16



2mg DNA 4x every 3 wks

DNA immunizations (n=5):

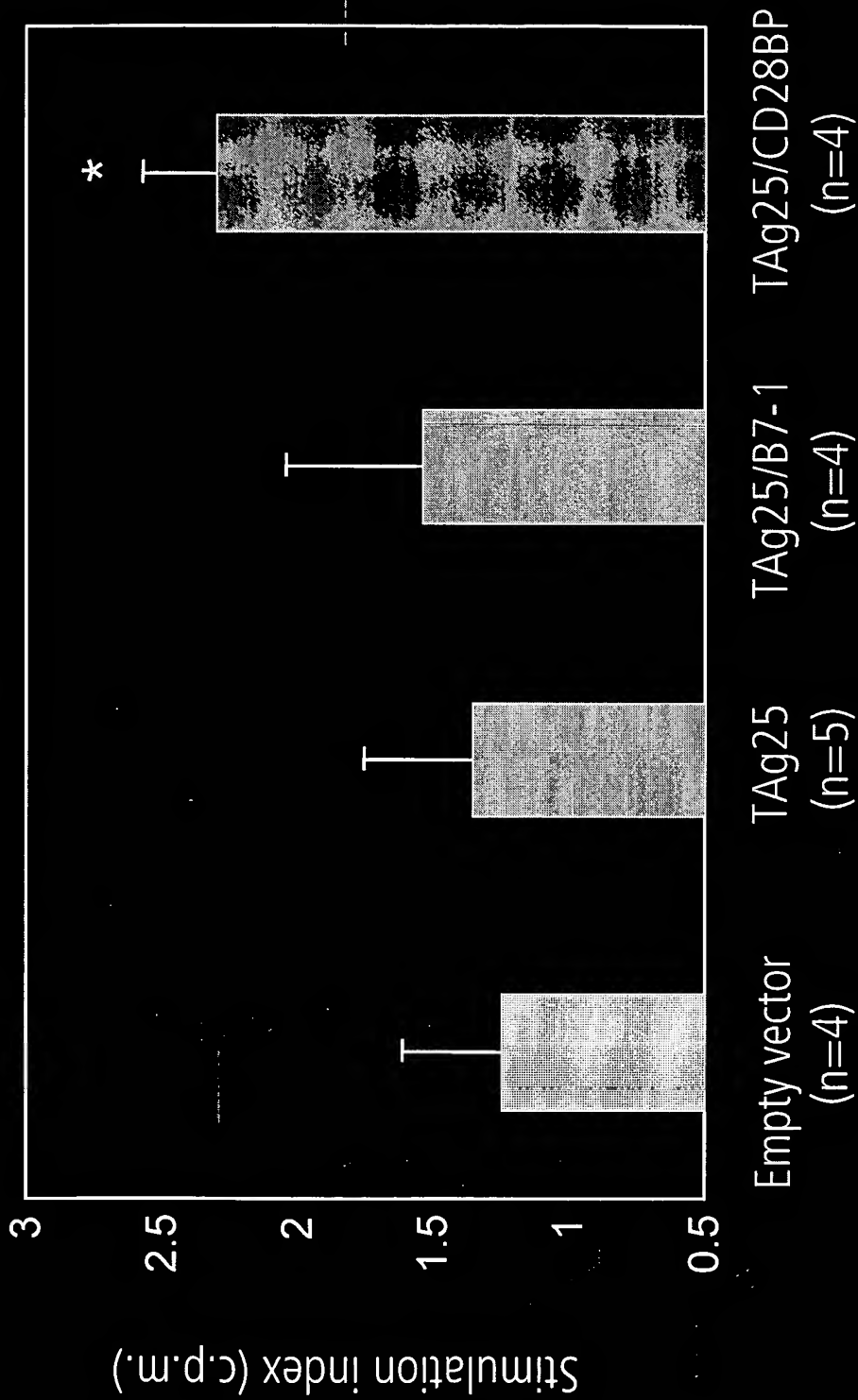
- Empty vector i.m. or i.d.
- TAg25 i.m. or i.d.
- TAg25/hB7.1 i.m. or i.d.
- TAg25/CD28BP i.m. or i.d.
- TAg25 + control DNA i.m.
- TAg25 + hB7.1 i.m.
- TAg25 + CD28BP i.m.

100ug TAg25 protein in 2mg Alum
 2x every 4wks; i.m.
 Given to 20 monkeys previously
 treated with DNA i.d.



Animal Species:
 Macaca fascicularis

Figure 17



Immunization i.d.
Restimulation using a mixture of 9-11 $\alpha\alpha$ EpCAM-specific peptides

Figure 18

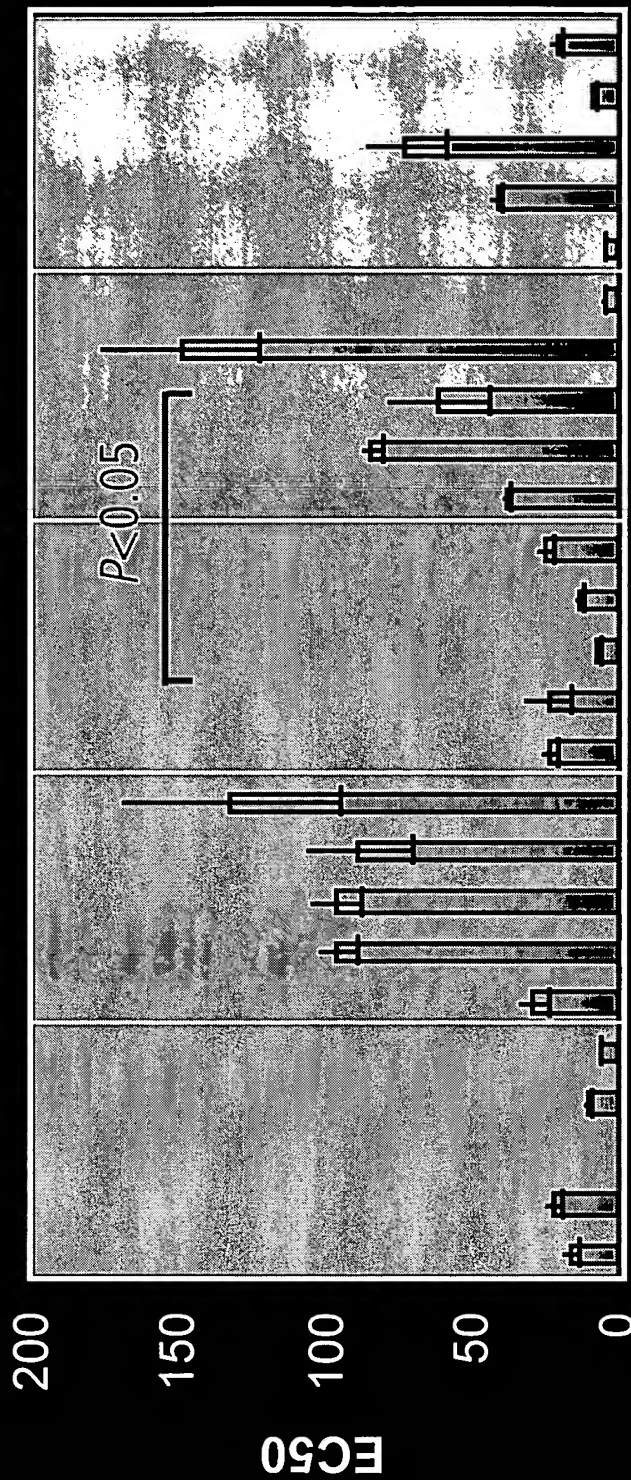
Antigen-specific Response

Vaccine	CD4 T Cell *	CD4 & CD8 T Cell **
Empty vector	0/5	0/5
TAg25	1/5	0/5
TAg25/B7-1	0/5	0/5
TAg25/CD28BP	3/5	2/5

* Number of animals positive when restimulated with TAg25

** Number of animals that are positive for restimulation with both TAg25 and EpCAM peptide mixture in separate cultures
 > 10 spots above background is considered positive

Figure 19



Immunization: Empty vector TAg25 TAg25/hB7.1 TAg25 + CD28BP TAg25 + control DNA

Dose: 2mg 2mg 2mg 1mg + 1mg

4th DNA

Figure 20

